Allosensitization following bone graft

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Abbreviations

cRF, calculated reaction frequency

DSA, donor specific antibody

PRA, panel reactive antibody

ABSTRACT

It is recognised that patients may become sensitized to donor-specific HLA antigens as a result of previous antigenic exposures, classically through previous transplantation, pregnancy or blood transfusion. We present an unusual case of a patient who unexpectedly developed a range of anti-HLA antibodies following orthopaedic surgery where a bone graft was deployed intraoperatively.

We describe the case of a 52-year-old male awaiting a renal transplantation, undergoing elective orthopaedic surgery requiring a small volume bone graft. His post operative antibody profile was found to be substantially changed compared to his previous negative samples, with the presence of HLA-DR, DQ and DP specificities, at levels that would be likely to give a positive flow cytometry

crossmatch and therefore according to local procedures required listing as unacceptable antigens for organ allocation. We perform a literature review of all previous cases of allosensitization following bone graft.

This case is the first to demonstrate allosensitization following minor surgery with low volume bone graft. Previous evidence is very limited and pertains only to massive osteochondral surgery for trauma or malignancy, and is confounded by potential concomitant blood transfusion. Clinicians should be aware of the risk of allosensitization where bone grafts are used.

Introduction

It is well recognised that patients may become sensitized to donor-specific HLA antigens as a result of previous antigenic exposures, classically through previous transplantation, pregnancy or blood transfusion. The development of anti HLA antibodies has important implications for subsequent time spent on transplant waiting lists as highly sensitized patients are more difficult to match with a donor organ. Furthermore, the presence of anti-HLA antibodies at the time of transplant , particularly donor specific antibodies (DSA), is correlated with poorer long term renal transplant survival.(1,2)

In the United States 20,000 patients awaiting a renal transplant are considered highly sensitized and these patients subsequently spend longer on the waiting list than those without donor specific antibodies.(3,4) These highly sensitized patients constitute approximately 10% of all active deceased renal transplants recorded in the UNOS registry.(5)

Thus, minimisation of the development of anti-HLA antibodies is of vital importance to potential transplant recipients and clinical practice should attempt to mitigate these risks wherever possible.

Bone grafts are traditionally thought to represent a low immunological risk of alloimmunization, perhaps due to uncertainty surrounding viability of remaining marrow and antigen presenting cells in graft material. However, there is a small body of evidence beginning to accumulate which suggests a previously unrecognised risk is associated with bone grafts, which may be clinically important for some patients.(6) We present an unusual case of a patient who unexpectedly developed a broad range of anti-HLA antibodies following orthopaedic surgery where a bone graft was deployed as part of the intraoperative technique.

The Case

A 52-year-old male had spent 6 months on the waiting list for a deceased donor kidney transplant when he was admitted for a right sided medial opening wedge high tibial osteotomy for symptomatic medial compartment osteoarthritis in June 2016.

His primary renal diagnosis was focal segmental global sclerosis secondary to chronic IgA nephropathy which had presented as acute nephritic syndrome seven years prior. This had progressed in the context of heavy proteinuria until he had commenced haemodialysis two years prior to admission.

His dialysis history was uneventful. He dialysed through a tunnelled central vascular catheter three times a week. His sessions were well tolerated, he had never had any dialysis associated infections and his treatment adequacy and biochemical control

were excellent. He had never required any blood transfusions, his haemoglobin being well maintained instead by twice weekly subcutaneous erythropoietin beta.

The patient had undergone an identical operation in his opposite knee two years prior to this procedure to good effect and he continued working in an active job in the catering industry.

His HLA antibody profile was established during his evaluation prior to placement onto the transplant waiting list. Importantly, prior to his orthopaedic surgery he had no detectable anti-HLA antibodies.

His surgical course was uncomplicated. Intraoperative blood loss was minimal and no blood products were administed at any point. In order to improve stability and promote healing at the osteotomy site the operating team elected to deploy two wedges of femoral head allograft bone graft in addition to the osteotomy plate. The estimated volume of bone graft used was $2cm^3$.This was fresh frozen bone supplied by the national bone bank. Our local protocol involves donor screening for blood borne virus testing and for blood group (allowing issue of RhD negative bone to recipient females of child bearing potential who are RhD negative). A small section of bone is removed for culture and the bone is then immediately stored fresh frozen at -80°C (-112 °F) for up to 3 years. Bone is supplied unwashed to theatre, where surgical preference dictates whether bone is washed. The bone used in this case was not washed.

He contined to attend his haemodiaylsis post operatively and was discharged home following a brief in-patient stay.

Five weeks following his surgery, a routine antibody profile update was performed. His antibody profile was found to be substantially changed compared to his previous negative samples, with the presence of HLA class II antibodies (figure 1). Single antigen bead array analysis (One Lambda and Immucor) using the Luminex platform showed the presence of HLA-DR, DQ and DP specificities, at levels that would be likely to give a positive flow cytometry crossmatch and therefore according to local procedures required listing as unacceptable antigens for organ allocation. The calculated reaction frequency (cRF) level was 99%.

DNA from the bone graft donor was extracted from a residual plasma sample and HLA typed using Luminex SSO (One Lambda). The HLA types of the patient and the bone donor showed a 1,1,2 mismatch for HLA-A,B and DR. Allele level donor and patient HLA types were imputed from the SSO data and with the patient HLA antibody data were used in an epitope analysis (Matchmaker) to assess the likelihood of the bone donor being the cause of patient sensitisation (Figure 1). The results showed the presence of antibodies directed against mismatched donor HLA epitopes. Repeat testing six months following the procedure demonstrated persistance of the class II reactivity, although the median florescent intensity values were noted to have decreased.

Discussion

Bone Grafting

Bone grafting is a common orthopaedic procedure performed to augment post-operative bone regeneration. An autologous bone graft remains the gold standard and common harvesting sites include the iliac crest and intramedullary canal of long bones.(7,8) However, it is well recognised that harvesting of autologous bone graft is associated with an increase in postoperative pain and donor site morbidity.

Alternatives to autologous graft include allograft bone graft, allograft demineralized bone matrix and synthetic material (e.g. tricalcium phosphate or hydroxyapatite).

Bone allografts are kept in a local hospital banks or national bone banks. The primary source of bone allograft is femoral heads, donated by patients following hip arthroplasty. Bone grafts may also be donated by deceased donors.(9)

The method of processing depends on the specific bank but can vary from the graft being used fresh, freeze dried or frozen. Many are transplanted without further processing, but protocols do exist for allograft “washing”. These protocols may include various degrees of heat treatment, the use of ethylene oxide sterilisation and gamma radiation. Over 95% of leukocytes and plasma components, as measured by elastase and soluble protein, can be removed in such a manner.(10) These protocols are primarily driven by infectious concerns rather than any immunological considerations.(10,11) Despite this, animal models suggest that frozen and freeze dried bone transplants are less immunogenic than fresh bone and have more successful engraftment.(12)

During the normal healing process of a bone allograft, revascularization and osteoclastic activity are thought to continuously replace the cells of the allograft with host bone. This cellular invasion and neovascularisation does have some similarities to elements of transplant rejection, leading some authors to question the applicability of traditional concepts of rejection to bone grafts.(13)

Previous clinical experience

Allograft bone procedures are performed without any HLA matching or immunosuppression protocols. This is considered clinically unnecessary given that clinical rejection is extremely rare, although it has been reported to occur.(14)

Despite early evidence to the contrary, it has been noted that the overall anti HLA antibody profile of patients can be altered following bone graft donation, although there has been a paucity of data specifically measuring anti-HLA antibodies outside of massive osteochondral transplants.(15,16)

Evidence that alloimmunization may occur comes from a multicentre prospective study of patients receiving cortex-replacing, massive structural bone allografts. It was noted that donor-specific HLA sensitization occurred in 57% of the patients but subsequently had no demonstrable effect on bone graft incorporation or union.(17)

A second prospective study population demonstrated massive bone transplantation operations were associated with donor-specific HLA sensitization in 53% of previously nonsensitized patients.(18) Both studies pertained to bone transplant on a much larger scale than our case - massive osteochondral grafts due to trauma or malignancy, with consequently larger antigenic loads, more varied antigen exposures, and were potentially confounded by coexistent bloods transfusions. Such

observational studies do serve as a proof of concept that bone grafts can generate a clinically significant response, but protein characterization of the immunoreactive proteins revealed that the majority of antigenic targets were fragments of various collagen molecules.(19)

Specific cases relating to HLA sensitisation that may inform our practice within clinical transplant medicine are very limited. Following a total knee arthroplasty to treat osteosarcoma and composite bone allograft prosthesis a potential kidney transplant recipient's PRA rose from 28% to a peak of 70%.(20) A second report of a patient developing DSA, also following osteosarcoma resection and tibial reconstruction with allogenic bone graft has been reported. While this patient had a concomitant blood transfusion, it is possible that the large quantity of bone used was a factor in inducing allosensitization.(6)

Our case represents the first description of allosensitization following a simple bone graft with a very small volume of donor bone used and adds to a small but significant body of evidence surrounding the immunology of bone grafts. This is an interesting observation as it is expected that there would be few HLA class II positive cells in the graft. One potential source of HLA class II positive cells in a bone graft could include residual bone marrow which could include dendritic cells, macrophages and B-cells. Furthermore, recent evidence has demonstrated that crosstalk between the immune system and cells of bone lineage is more common that previously recognised. Osteoblasts have been noted to express MHC class II surface proteins and act as antigen presenting cells.(21) Additionally data suggest a large proportion of osteocytes die following bone grafting, which may explain the why allosensitization via these cells is far less common than one might expect.(22)

Conclusions

When planning orthopaedic surgery for potential transplant recipients, clinicians should be aware of the risk of allosensitization where bone grafts are used. These may not be immediately recognised as a potential source of antigenic exposure, but the lack of HLA matching and immunosuppression when they are used can prove to be a source of sensitisation. Furthermore, decisions surrounding the use of donor bone may not be entirely predictable as individual surgical teams may need to unexpectedly consider bone grafting intraoperatively.

Pragmatically, consideration should be given to washing bone to reduce the antigenic load or to the use of osteoconductive alternatives to bone grafts if appropriate. This would include synthetic materials such as hydroxyapatite and calcium phosphate cements. These materials are useful in providing structural support after osteotomy and other orthopaedic procedures but have no risk of sensitization as there is no antigenic component. Other alternatives include osteoinductive materials of which demineralized bone matrix has been the most commonly used. This is a particulate powder in a carrier putty composed of 93% collagen, 5% soluble osteoinductive proteins and 2% residual mineralized matrix.(23,24) Importantly, this still has potential for alloimmunization given the potential antigenic load of protein and bone matrix.

Finally, increasingly diverse tissues are now transplanted routinely, including hands, vessels, nerves, skin, cartilage, tendons and muscle. As with bone, these procedures should all be considered as potential sources of alloimmunization in patients awaiting solid organ transplant, and their exposure to such sources should be minimised where practical and possible.

Disclosure:

The authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

Figure Legend

Figure 1: Patient and donor HLA types were determined by Luminex SSO typing and inputted into HLAMatchmaker allowing determination of mismatched class II epitopes. (HLA class I epitope mismatches were not determined as patient remained class I antibody negative). Mismatched alleles to which antibody was generated are highlighted in boxes. Analysis of reactive serum post orthopaedic surgery demonstrates possible reactivity with 6 of the mismatched HLA class II epitopes across HLA-DRB1, DQB1 and DPB1 alleles. Reactivity is shown in descending order of MFI, strongest reactivity directed towards DQ6 alleles with epitope 52PQ2 demonstrating the strongest levels in terms of MFI (52PQ2 actually indicates two separate configurations 52P53Q and 84E85V in opposite locations on the top of the DQB molecule). Interestingly reactivity appears to have been generated towards all HLA-DR, DQ and DP loci. Antibody reactive epitopes listed as confirmed in the HLA epitope registry are shown in bold in the table; the other antibody reactive epitopes are listed as provisional in the database.(25)

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Patients HLA type: A*02:01, 11:01; B*44:02, 57:01; C*05:01, 03:03; DRB1*07:01, 12:02; DRB3*02, DRB4*01:03:01:02N; DQB1*03:01, 03:03; DPB1*02:01; DPA1*01:03

Patient HLA antibody status: - **pre** orthopaedic surgery: Class I Neg, Class II Neg
post orthopaedic surgery: Class I Neg, Class II **Pos**

Immunizer HLA type: A*01:01; B*18:01; C*07:01; DRB1*11:04, 15:01; DRB3*02; DRB5*01; DQB1*03:01 06:02; DQA1*01:02, 05:05; DPB1*14:01, 19:01; DPA1*02:01, 02:02

Mismatched Class II Epitopes: **DRB1*11:04**, *11STS*, *31FYY, 37YV*, *57DE, 57DEDP, 28D, 28DY, 30Y, 58EEDP, 85V, 85VV, 86V, 96H, 98K, 98KS, 104S, 108P, 112H, 120S, 140T, 140TV, 149H, 180V, 181T*; **DRB1*15:01,** *142M*, *370QT, 28D, 28DY, 30Y, 37S, 67IQ, 70QA, 71A, 85V, 85VV, 86V, 96Q, 98K, 98KS, 104S, 108P, 112H, 120S, 140A, 149Q, 180V, 181T*; **DQB1*06:02,** ***52PQ2, 52PR***, *85VA, 87F, 140A2*; **DRB5*01,** *96EV, 108T, 133RS, 28H, 31I, 37D2, 85V, 86G, 96EN3, 98K, 98KN, 112H, 120N, 140A, 140AV, 149Q, 180V, 181T*; **DPB1*14:01,** *57D, 84DEAV, 96K, 8V, 9H, 11L, 65L, 65LK, 76V*; **DPB1*14:01,** *84DEAV, 96K, 55EA, 76I*

HLA Allele		MFI	Antibody reactive mismatched HLA epitopes					
B-chain	α-chain	Value	Epitope 1	Epitope 2	Epitope 3	Epitope 4	Epitope 5	Epitope 6
DQB1*06:09	DQA1*01:02	20165	**52PQ2**	**52PR**				
DQB1*06:03	DQA1*01:03	19708	**52PQ2**	**52PR**				
DQB1*05:01	DQA1*01:01	18607	**52PQ2**	**52PR**				
DQB1*06:01	DQA1*01:03	18244	**52PQ2**	**52PR**				
DRB1*14:01	-	16862			11STS			
DRB1*13:03	-	16064			11STS	31FYY37YV		
DRB1*03:01	-	15345			11STS			
DRB1*11:04	-	15224			11STS	31FYY37YV		
DRB1*14:54	-	14561			11STS			
DRB1*03:02	-	14547			11STS			
DQB1*06:02	DQA1*01:01	14291	**52PQ2**	**52PR**				
DRB1*13:01	-	14229			11STS			
DQB1*06:02	DQA1*01:02	13568	**52PQ2**	**52PR**				
DQB1*06:04	DQA1*01:02	13968	**52PQ2**	**52PR**				
DRB1*11:01	-	13451			11STS	31FYY37YV		
DRB1*14:02	-	12571			11STS			
DQB1*05:02	DQA1*01:02	11326	**52PQ2**	**52PR**				
DRB1*04:01	-	9098				31FYY37YV	-	
DRB1*04:03	-	9079				31FYY37YV		
DQB1*04:01	DQA1*03:03	8901		**52PR**				
DRB1*04:05	-	8719				31FYY37YV		
DRB1*04:02	-	7934				31FYY37YV		
DPB1*05:01	DPA1*02:01	6607						**84DEAV, 96K**
DPB1*01:01	DPA1*02:02	6575						**84DEAV, 96K**
DPB1*14:01	DPA1*02:01	6429						**84DEAV, 96K**
DRB1*08:01	-	6425				31FYY37YV		
DPB1*01:01	DPA1*02:01	6398						**84DEAV, 96K**
DPB1*13:01	DPA1*02:01	6390						**84DEAV, 96K**
DPB1*19:01	DPA1*02:01	6361						**84DEAV, 96K**
DPB1*17:01	DPA1*02:01	6309						**84DEAV, 96K**
DRB1*15:01	-	6098					**142M3**	
DRB1*16:01	-	6045					**142M3**	
DPB1*05:01	DPA1*02:02	6040						**84DEAV, 96K**
DQB1*04:02	DQA1*04:01	5958		**52PR**				
DRB1*16:02	-	5908					**142M3**	
DRB1*1502	-	5622					**142M3**	
DQB1*04:02	DQA1*02:01	4829		**52PR**				
DPB1*13:01	DPA1*04:01	4068						**84DEAV, 96K**
DQB1*04:01	DQA1*02:01	3485		**52PR**				
DPB1*03:01	DPA1*01:03	2765						**84DEAV, 96K**
DPB1*01:01	DPA1*01:03	2348						**84DEAV, 96K**
DPB1*01:01	DPA1*03:01	2304						**84DEAV, 96K**